

AD _____

COOPERATIVE AGREEMENT DAMD17-96-2-6025

TITLE: Determination of Total Daily Energy Requirements and Activity Patterns of Service Women.

PRINCIPAL INVESTIGATOR: James P. Delany, Ph.D.

CONTRACTING ORGANIZATION: Louisiana State University
Pennington Biomedical Research Center
Baton Rouge, Louisiana 70808

REPORT DATE: October 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

DTIC QUALITY INSPECTED 2

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 1998	3. REPORT TYPE AND DATES COVERED Annual (26 Sep 97 - 25 Sep 98)	
4. TITLE AND SUBTITLE Determination of Total Daily Energy Requirements and Activity Patterns of Service Women			5. FUNDING NUMBERS DAMD17-96-2-6025	
6. AUTHOR(S) James P. Delany, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Louisiana State University Pennington Biomedical Research Center Baton Rouge, Louisiana 70808			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, Maryland 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) The objective of the current study is to define a range of energy requirements of servicewomen, defining the variation as it relates to jobs, military settings, and activity patterns. This is crucial information needed not only for determination of nutritional requirements for energy balance, but specific nutrient density standards for servicewomen. Total daily energy expenditure is measured using the doubly labeled water (DLW) method. Activity patterns from actigraphs will be analyzed for hours of sleep, description of job/work patterns by examining bursts of concerted activity versus steady activity. Men will also be studied in these settings. Energy requirements for men have been better established and will serve to anchor the results obtained in women to previously established norms in men. Several field studies will be conducted over the course of the grant. The first field study was conducted at Fort Bragg/Camp Mckall during a Combat Support Hospital training exercise. Energy expenditures were moderate, and higher in men than women. However, when adjusting for differences in body size, energy expenditures were similar. Very high energy expenditures were observed during the Crucible studies in Marine Recruits. Further data analyses for this study are being carried out. Planning for the next studies are underway.				
14. SUBJECT TERMS Defense Women's Health Research Program Energy requirements, doubly labeled water, physical activity patterns energy expenditure, military nutrition, hydration status.			15. NUMBER OF PAGES 28	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

19990407 091

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

FOREWORD

JPD Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

JPD For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

James P. Delany
PI - Signature

10-23-98
Date

4. TABLE OF CONTENTS

1.	SF 298 Report Documentation Page	2
2.	Foreword	3
3.	Table of Contents	4
4.	Introduction	5
5.	Body	6
6.	Conclusions	22
7.	References	23
8.	Appendices	24

5. INTRODUCTION

Women comprise 12.3% of the U.S. military active duty personnel, or approximately 200,000 servicewomen (as of June 30, 1993) (1). This is a significant number even compared to the 1,518,752 active duty men in military service, yet nutritional requirements of women have been far less studied than for men.

Energy Requirements in Women

Although energy requirements of male soldiers have been and continue to be assessed by our labs (USARIEM and PBRC) and others under several environmental and training conditions using the doubly labeled water (DLW) method, energy requirements of female military personnel have not been studied.

Several studies which have included a subset of female subjects, have examined nutrient intake, which may give some idea of energy requirements. A recent assessment of intake was made in 49 Army women by a visual estimation method during an 8-week cycle of the Army Basic Combat Training course (2). Reported intake was 2592 ± 500 kcal/d, which was within the range of energy intakes of 2000 - 2800 kcal/d for female soldiers ages 17-50 years old as defined by the Military Recommended Dietary Allowances MRDA (3). However, the range of intakes ranged from a low of 1294 to a high of 4388 kcal/d. Some of this is certainly due to errors in estimating energy intake, while some is due to true variations in intake. Energy deficit based on body composition changes averaged 180 kcal/d suggesting energy expenditures as high as 2800 kcal/d (4). Consumption of several micronutrients were less than adequate. Vitamin B₆ (76%), Folic acid (65%), calcium (73%), zinc (73%) and iron (90%) were each consumed at levels lower than that of the MRDA. These inadequate intakes point to a potential problem women may encounter when consuming military field rations. The nutrient density of these rations was designed with the higher energy requirements of males. A female recruit consuming meal ready to eat (MRE)s at an expenditure level of 2400 kcal/d would need to consume 131% of energy requirements to meet her daily needs for calcium and as high as 166% of energy requirements to meet her daily needs of iron. It may be necessary to supplement the rations with specific micronutrients to be used by those with lower energy intake requirements or design specific rations for smaller women soldiers.

The objective of the current study is to define a range of energy requirements of servicewomen, defining the variation (with adjustments made for body size/composition) as it relates to jobs, military settings, and activity patterns. This is crucial information needed not only for determination of nutritional requirements for energy balance, but specific nutrient density standards for servicewomen. This will address the first and third specific nutrition topics of the IOM report. Total daily energy expenditure will be measured using the doubly labeled water (DLW) method. As part of the DLW method, total water turnover can be calculated from deuterium elimination and total body water. Corrections are made for atmospheric water exchange, metabolic water and isotopic fractionation. From these calculations we can estimate actual fluid consumption in the field (the second nutrition topic) and fluid requirements during

specific categories of jobs and tasks (third nutrition topic). Activity patterns from actigraphs will be analyzed for hours of sleep, description of job/work patterns by examining bursts of concerted activity versus steady activity. Activity patterns will also be assessed using a boot insert which measures locomotory activity and voluntary energy expenditure. Men will also be studied in many of these settings. Energy requirements for men have been better established and will serve to anchor the results obtained in women to previously established norms in men (or confirm the validity of significant deviations also observed in the female data). We hypothesize that in some settings, there may be smaller differences between genders (normalized for fat free mass (FFM)) than in Army basic training, if absolute rather than relative, or ability group standards are emphasized. Such a finding would help demonstrate and explain a wider possible variation in female energy requirements.

6. BODY

METHODS

Participants

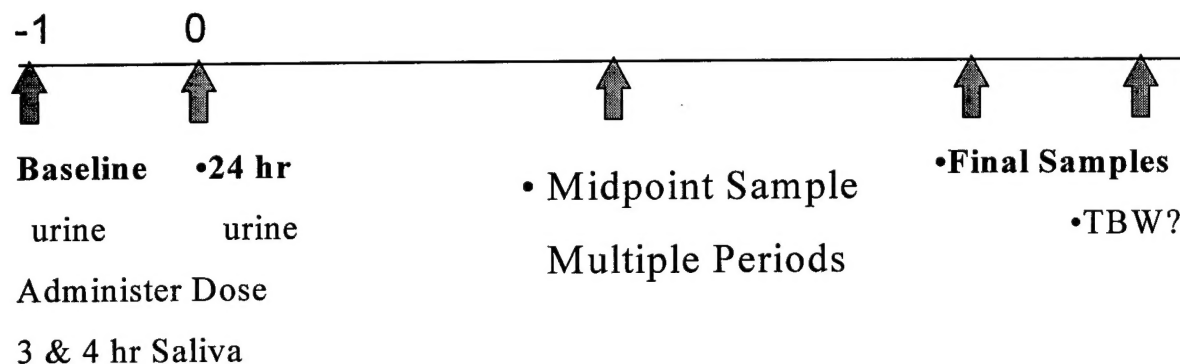
Subjects for these studies, with respect to gender, are as described above. We will attempt to recruit subjects to reflect the racial and ethnic makeup of the military units. This will generally include about 40% white and 40% black enrollment.

Inclusion Criteria would dictate that the volunteers be: within an age range is 18-35 inclusive, within 2 standard deviations for height and weight, female subjects must be non-pregnant and non-lactating, must be healthy with no pre-existing medical conditions, must be on no regular medication, must be able to communicate meaningfully with the investigator, be legally competent to give written and informed consent.

Exclusion Criteria would include volunteers with: Medical history of cancer, heart, kidney, liver disease or HIV or any other chronic or acute condition, thyroid dysfunction or any other significant endocrine abnormality, weight loss or gain greater than 10 pounds within the preceding two months.

DLW

A typical DLW protocol is depicted in the Figure below. The study begins with the collection of baseline urine and/or saliva samples, followed by oral administration of the $^2\text{H}_2^{18}\text{O}$ dose. Saliva samples are obtained 2-4 hours after the dose for calculation of dilution spaces (5). A urine sample is collected the following morning for measurement of initial enrichment. Urine samples are then collected at the end of the period for measurement of final isotopic enrichment. The length of time a DLW study can be carried out depends on the turnover of the two isotopes, which is dependent on water and CO_2 output. For studies in typical adults, the optimal metabolic period is 4-21 days (6), with Military Nutrition studies generally limited to the shorter time periods. If total body water (TBW) is expected to change over the metabolic period, subjects will need to be redosed with deuterium oxide at the end of the study for a final TBW measurement.



Since the DLW dose and the analyses are so expensive it is imperative that one obtains enough specimens to have backup samples at the beginning and end of the period of interest. Problems with specimens can occur in the field or in the lab, such as inability to obtain a specimen, loss of a specimen or contamination of a specimen. The protocol depicted in Figure 2 above illustrates the minimum number of specimens to be collected for a DLW study. This protocol provides backup samples for the initial and final time points. If there is a problem with the 24-hr urine, the 4 hr saliva specimen can be used as the initial time point. If the final sample is bad the urine from the previous day can be used as the final enrichment for elimination rate calculations.

The ^{18}O isotope abundances will be measured on a Finnigan MAT 252 gas-inlet Isotope Ratio Mass Spectrometer with a CO_2 -Water equilibration device (7). Briefly, urine and saliva samples will be equilibrated with CO_2 at 18 degrees C in a shaking water bath for at least 8 h. The CO_2 is then cryogenically purified under vacuum before introduction into the mass spectrometer. The hydrogen isotope abundances will be measured on a Finnigan MAT 252 gas-inlet Isotope Ratio mass Spectrometer, as previously described (7). Briefly, urine and saliva samples will be distilled under vacuum into Vycor tubes containing zinc reagent (Friends of Biogeochemistry, Bloomington, Indiana). The reduction tube will be sealed with a flame and placed in a 500 °C oven for 30 minutes to reduce the water to hydrogen gas which will then be introduced into the mass spectrometer. The ^2H and ^{18}O isotope elimination rates (k_{H} and k_{O}) will be calculated using the isotopic enrichment relative to predose of the first day and each of the last two days of the metabolic study. During field studies, corrections for baseline isotope shifts from changes in water supply will be made by following a group not receiving isotope. Energy expenditure is calculated by multiplying rCO_2 by the energy equivalent of CO_2 for an assumed respiratory quotient (RQ) of 0.83 or that calculated from the food quotient (FQ) of the rations consumed and estimated changes in body energy stores during the study, as previously described (7).

The ^2H and ^{18}O isotope elimination rates (k_{H} and k_{O}) can be calculated by the two-point method using the initial (i) isotopic enrichment and the final (f) enrichments: $k = (\ln \text{atom percent excess (APE)}_i - \ln \text{APE}_f) / \Delta t$. CO_2 production will be calculated according to Schoeller et al. (6) as recently modified (8):

$$\text{rCO}_2 = (\text{N}/2.078)(1.007k_{\text{O}} - 1.041k_{\text{H}}) - 0.0246\text{rH}_2\text{O}_f$$

where N is the total body water calculated from the ^{18}O enrichment in the 4 hour saliva (or average of initial and final 4 hr saliva samples if TBW is expected to change), and $r\text{H}_2\text{O}_f$ is the rate of fractionated evaporative water loss which is estimated to be $1.05N(1.007k_o - 1.041k_H)$.

Body Composition

Height will be measured with a calibrated stadiometer on subjects in stocking feet and with heads positioned in the Frankfort plane. Body weights will be obtained from subjects in gym shorts and T-shirt using a calibrated electronic balance.

Body circumferences will be measured in accordance with the gender-specific methods of the Army, Navy, and Marine Corps, in every subject. (The Air Force is currently using the Navy equations.) Body fat calculations will be made for each of the service equations.

Body composition will be measured from measurement of body water from the doubly-labeled water technique to estimate fat-free mass in the subjects, using the fixed value of 0.73 for fat-free mass hydration.

Foot Strike Monitor

Laboratory validation study design. Paralleling our earlier validation study (9), twenty military-eligible female volunteers will randomly divided into two equal groups: an equation derivation group, and an equation validation group. The two groups will be similar in age, height, and weight. The derivation group will be used to generate an equation describing the relationship between measured loco, assessed by indirect calorimetry, and the ratio of body weight to time of contact of the foot on the ground (Wb/tc). This equation will be used to estimate loco from Wb/tc in a second group of subjects (the validation group). The validity of the equation will be tested by comparing measured loco and estimated loco.

Field training exercise (FTX) study design. Investigate the loco in subsets of women soldiers as a function of their natural military work environment. The body composition of each volunteer will be determined by anthropometry and isotope dilution immediately before and after about an eleven day FTX.

Activity Monitors

Motionlogger Actigraphs, model AMA-32 (Precision Control Devices, Ft. Walton Beach, Fl.) will be employed to assess patterns of rest and activity, total physical activity and to estimate duration and fragmentation of sleep. This will permit comparison of these parameters across the various groups tested. These monitors have been used in previous military energy expenditure field studies to provide information on individual and group physical activity levels and patterns of activity and sleep in volunteers (10). Of particular interest will be gender differences in total physical activity and daily patterns of rest and activity. Such differences have previously been observed in healthy young men and women engaged in similar activities (11). The devices are 4 cm L x 3.1 cm W x 1 cm H, weigh 57 g and are worn on the wrist of the non-preferred hand using a standard wristwatch band. Each device contains a microcomputer, 32 k of memory, an analog-to-digital (A/D) converter and a piezoelectric sensor. They are powered by standard

wristwatch batteries and can record continuously for over a week. Due to their small size and similarity to a wristwatch subjects do not find wearing them objectionable in any way. To obtain this high level of fidelity the monitors sample total activity counts in one-minute blocks of time. Information on sleep patterns in the groups we propose to test is not currently available. Data collected by the AMA-32 will be downloaded to a laptop computer for further analysis using the ACTION 3 computer program (Ambulatory Monitoring, Inc.; Ardsley, NY).

TECHNICAL OBJECTIVES

KEY OBJECTIVES

- I. Define energy expenditure in servicewomen in various military settings.
- II. Determine if differences in total daily energy expenditure (TDEE) are explained primarily by differences in body size and fat-free mass after differences in activity patterns (locomotory and by wrist-worn actigraphy) are accounted for.
- III. Determine if the same holds true for differences between typical men, small men, and women.
- IV. Test methods which may be useful in prediction of TDEE.
- V. Assess hydration status of men and women by deuterium turnover (part of DLW).
- VI. Compare TDEE assessed by footstrike monitor to DLW.
 - A. Laboratory study: Demonstrate that the foot contact monitor (FCM) method provides valid estimates of the loco in military-eligible women over a full range of walking and running speeds, regardless of the phase of the menstrual cycle.
 - B. Field study: Establish the validity of estimates of total daily energy expenditure (estimated TDEE), calculated from FCM determinations of loco and resting metabolic rate, in female soldiers engaged in military training at the Marine Corps Mountain Warfare Training Center (MCMWTC), Bridgeport, California. The doubly labeled water measurements of TDEE will serve as a reference standard (measured TDEE).

We hypothesize that estimates of total daily energy expenditure of women soldiers in the field (estimated TDEE) will provide valid estimates of actual TDEE (measured TDEE). Valid estimates of TDEE by the Foot Contact Monitor/Resting Metabolic Rate method would suggest that minute-to-minute loco data can be used to estimate macronutrient requirements associated with military training in mountainous terrain. This type of information is urgently needed to improve the match between macronutrient demand and macronutrient availability from rations and body energy stores.

STATEMENT OF WORK

Technical Objective: Determination Of Total Daily Energy Requirements, Water Turnover, and Activity Patterns of Servicewomen in Various Military Settings and Jobs

- I. Months 1-2: Preparation Phase
 - A. Protocol Development
 - B. Contact and clearly define FTXs
 - C. Hire/Train Personnel
 - D. Order DLW dose for first year
 - E. Order Actigraphs and components for Foot Contact Monitor
 - F. Principal Investigators Meet to discuss and refine protocols

- II. Months 6-18: Army Basic Training Field Study
 - A. Coordination Trip
 - B. Recruitment Trip
 - C. DLW dose preparation and shipment
 - D. Study team arrive and set up for field study
 - E. Conduct Energy Expenditure and Activity Pattern Study
 - F. Study team ship back equipment and samples
 - G. Isotope Analyses
 - H. Report Preparation

- III. Months 11-23: Marine Basic Training Field Study
 - A. Coordination Trip
 - B. Recruitment Trip
 - C. DLW dose preparation and shipment
 - D. Study team arrive and set up for field study
 - E. Conduct Energy Expenditure and Activity Pattern Study
 - F. Study team ship back equipment and samples
 - G. Isotope Analyses
 - H. Report Preparation

- IV. Months 16-28: Mountain Warfare Training Field Study
 - A. Coordination Trip
 - B. Recruitment Trip
 - C. DLW dose preparation and shipment
 - D. Study team arrive and set up for field study
 - E. Conduct Energy Expenditure and Activity Pattern Study
 - F. Study team ship back equipment and samples
 - G. Isotope Analyses
 - H. Report Preparation

- V. Months 20-32: Shipboard Field Study
 - A. Coordination Trip

- B. Recruitment Trip
 - C. DLW dose preparation and shipment
 - D. Study team arrive and set up for field study
 - E. Conduct Energy Expenditure and Activity Pattern Study
 - F. Study team ship back equipment and samples
 - G. Isotope Analyses
 - H. Report Preparation
- VI. Months 25-36: Army Units Field Study
- A. Coordination Trip
 - B. Recruitment Trip
 - C. DLW dose preparation and shipment
 - D. Study team arrive and set up for field study
 - E. Conduct Energy Expenditure and Activity Pattern Study
 - F. Study team ship back equipment and samples
 - G. Isotope Analyses
 - H. Report Preparation
- VII. Months 34-36
Prepare Final Report

SUMMARY OF PROGRESS

- I. Months 1-2: First field training study identified, protocol developed, Personnel hired and trained, DLW dose water ordered, actigraphs ordered. We delayed purchasing new foot contact monitors as a new, improved version was being developed that is attached to the boot externally, so that we no longer have to have a custom boot insert made for the monitor. Therefore, for the first field training study, we used some of the old version that Reed Hoyt had on hand. We also delayed the validation study of the FCMs until the new version was received.
- II. Months 6-18: The first field study was conducted at Fort Bragg/Camp Mckall, NC, in a Combat Support Hospital field study. Isotope analyses and energy expenditure calculations have been completed. Actigraph data are being analyzed.
- III. Months 11-23: We were very fortunate that the opportunity arose to conduct energy expenditure studies in Marine Recruits undergoing the grueling Crucible event conducted at Parris Island, South Carolina. The USARIEM group was asked to conduct cold weather studies in January and February, and I was able to join the team as this project fit perfectly with the aims of this grant.
- IV. Months 16-28:
- A. We are currently in the process of working out the details of our shipboard activities. We are working with W. Keith Prusaczyk, M.S., Ph.D., a Research Physiologist at the Naval Health Research Center in San Diego, California. A meeting has been set up with Dr. Prusaczyk in San Diego, Dr. DeLany from

PBRC and Dr. Beverly Patton from USARIEM where initial details will be worked out.

- B. Discussions are also under way to possibly conduct studies during basic training at the Great Lakes Training facility and in the Marines at Parris Island.
- C. The new FCMs, which have been further revised to be attached to the boot laces, instead of on the side of the boot will arrive. We should receive some of these new devices shortly. The laboratory validation study will be conducted and they will be available for future studies.

FIRST FIELD TRAINING STUDY

This study was a combined effort of the Military Nutrition and Biochemistry Division, the Sustainability Directorate and the Science and Technology Directorate of the Natick Research, Development, & Engineering Center (NRDEC), and the Pennington Biomedical Research Center to assess the nutritional adequacy for women of the Meal, Ready-to-Eat ration during a field training exercise. The study occurred during the field training exercise of a combat service support unit and investigated gender differences in food selection, nutrient intake, and energy expenditure.

TEST VOLUNTEERS

Volunteers were recruited from the 28 Combat Support Hospital (CSH), Fort Bragg, that were engaging in a field training exercise of approximately 14-days duration starting on 1 May 1997. The CSH anticipated deploying almost half of its 520 personnel. This unit strength included 150 women, but did not include approximately 50 FORSCOM nurses that train with the unit. All soldiers from the unit who agreed to participate, except women who were pregnant, were included in the study.

Prior to the start of the study, the subjects were briefed on the nature and purpose of the study and the requirements for participation in the study and were familiarized with the experimental procedures. Subjects were informed verbally and in writing of their rights to withdraw from any part of the study without penalty or prejudice. The Commanding Officer of the prospective volunteers was informed of their responsibilities under AR 70-25 to ensure that the consent of any person under their authority to participate in this research is voluntary. Each subject completed a Volunteer Agreement Affidavit and Volunteer Subject Registry Data Sheet.

All volunteers were asked to participate in all data collection efforts. The volunteers were asked to complete questionnaires providing demographic information, medical history, diet history, nutrition knowledge and attitudes, to record all foods and fluids consumed for a total of seven days, and to record MRE lunches for an additional seven days. Individuals were asked to provide one blood sample and have body height taken once and body weights measured three times. A subsample of 32 volunteers were asked to participate in energy expenditure measures by a stable isotope technique and to wear wristband activity monitors and shoe liner foot contact monitors.

STUDY CONDITIONS

The experimental test period were occur during a routine field training exercise in a temperate environment. The soldiers were provided three MREs per day for seven consecutive days during the field exercise. They were requested to eat no food other than that provided by the study team; however, the investigators were not take any enforcement measures. The importance of this restriction were explained to the CSH personnel at the orientation briefing. Bulk beverages or hot water typically available to combat service support personnel in the field were allowed.

A qualified medical monitor was supplied by the unit and was available during the entire experimental period. The medical monitor was responsible for terminating a volunteer's participation if medically indicated. Appropriate emergency medical service was available at Fort Bragg at all times during all tests.

STUDY DESIGN

The data collection schedule is shown below. An orientation briefing was provided at the beginning of the study. Baseline assessments were conducted at this time. Baseline/descriptive measurements include: height, weight, body composition by skinfold measures, and blood chemistries. Demographics and nutrition knowledge questionnaires and the Diet Habit Survey were administered on the day of baseline measurements.

This collaborative study of women soldiers provided a unique opportunity to study their physiologic responses a multi-stress military training environment. The broad objectives were to: (1) quantitatively determine energy expenditure, and (2) use ambulatory monitoring technologies to make minute-to-minute measurements of soldier activity patterns and the metabolic cost of locomotion.

A. Test volunteers

20 women and 10 men dosed
1 woman and 1 man undosed

30 volunteers, 2/3 female and 1/3 males, received doubly labeled water (DLW). The remaining 2 volunteers served as placebo controls. These subjects collected urine samples (salvia samples not necessary) at the same time as those drinking the DLW dose. This allowed for a correction factor to be calculated for any changes in isotopic baseline that might occur. Subjects were selected to obtain a variety of job classifications (MOS).

B. Experimental design

This study had a repeated measures design in which each test volunteer serves as his own control. The experimental design is outlined in Fig. 1 below.

Figure 1. Schedule of measurements.

	Days																
	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MRE (+/- A-rations)			-	-	-	-	-	-	-	+	+	+	+	+	+	+	
Field training exercise	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
DLW/ $^2\text{H}_2^{18}\text{O}$ dose	x																
Saliva samples	x																
Urine samples	x	x	x						x	x	x		x		x	x	x
Food intake				x	x	x	x	x	x	x	x	x	x	x	x	x	
Body composition	x																x
Portable monitors*		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	

Note: DLW/ $^2\text{H}_2^{18}\text{O}$ dose = doubly labeled water, stable isotope labeled hydrogen and oxygen.

*Portable monitors record activity and metabolic cost of locomotion.

PROGRESS

1. Doubly Labeled Water

All urine and saliva samples for the 30 dosed subjects and the 2 placebo subjects have been cleaned and prepared for isotope analyses. Deuterium and ^{18}O analyses are complete. Final calculations of total body water (for EE calculations and for estimation of fat free mass), and total daily energy expenditures have been calculated. Subject characteristics and energy expenditure data are presented in the following table. As expected, the men were heavier, had a higher fat free mass (FFM) and had a higher energy expenditure. This was true over the whole period, as well as before the field training exercise (PreFTX) as well as during the FTX at Camp Mckall. As a first adjustment for the differences in body weight, energy expenditures were simply divided by body weight. When this was done, and this is not necessarily the most appropriate method of adjustment, but it is often done, there are no differences in energy expenditure between the men and women. As expected, energy expenditures during the FTX were higher than that observed pre-FTX.

Table 1. Subject characteristics and energy expenditure.

	Female	Male
	MEAN \pm SE	MEAN \pm SE
Age, y	27.2 \pm 1.5	28.4 \pm 2.5
Body Weight, kg	62.2 \pm 2.5	88.2 \pm 3.8
FFM, kg	45.7 \pm 1.5	70.5 \pm 2.2
Energy Expenditure, kcal/d		
PreFTX	2192 \pm 123	3246 \pm 180
FTX	2745 \pm 122	3959 \pm 159
Entire period	2677 \pm 114	3881 \pm 165
Energy expenditure, kcal/d divided by body weight		
PreFTX	37.8 \pm 2.1	35.6 \pm 1.4
FTX	44.9 \pm 1.6	45.5 \pm 2.2
Entire period	43.6 \pm 1.4	44.4 \pm 2.0

In addition to including women and men, subjects were selected to obtain a variety of job categories. Our original intent was to have similar breakdowns by job classification. However, we could not locate all of the subjects whom we had selected to obtain equal distributions (of those who had volunteered to participate in the study). We selected subjects from four major MOS groupings: (A) administrative; (M) medical which includes operating room specialists, practical nurses; (M1) Medical Specialists and Medical Lab Specialists; and (S) Utility Equipment Repair, Radio Operator, Medical Equipment Repair, Power Gen. Equipment Repair and Laundry Specialists. The numbers of each by gender, and the energy expenditures are given in the following table (Table 2). Energy expenditure was higher in men than women for each group. In addition, during the FTX, the lowest energy expenditures were observed in the administrative group.

Table 2. Pre-field training exercise (FTX) and FTX energy expenditures (kcal/d) by gender and job category groupings.

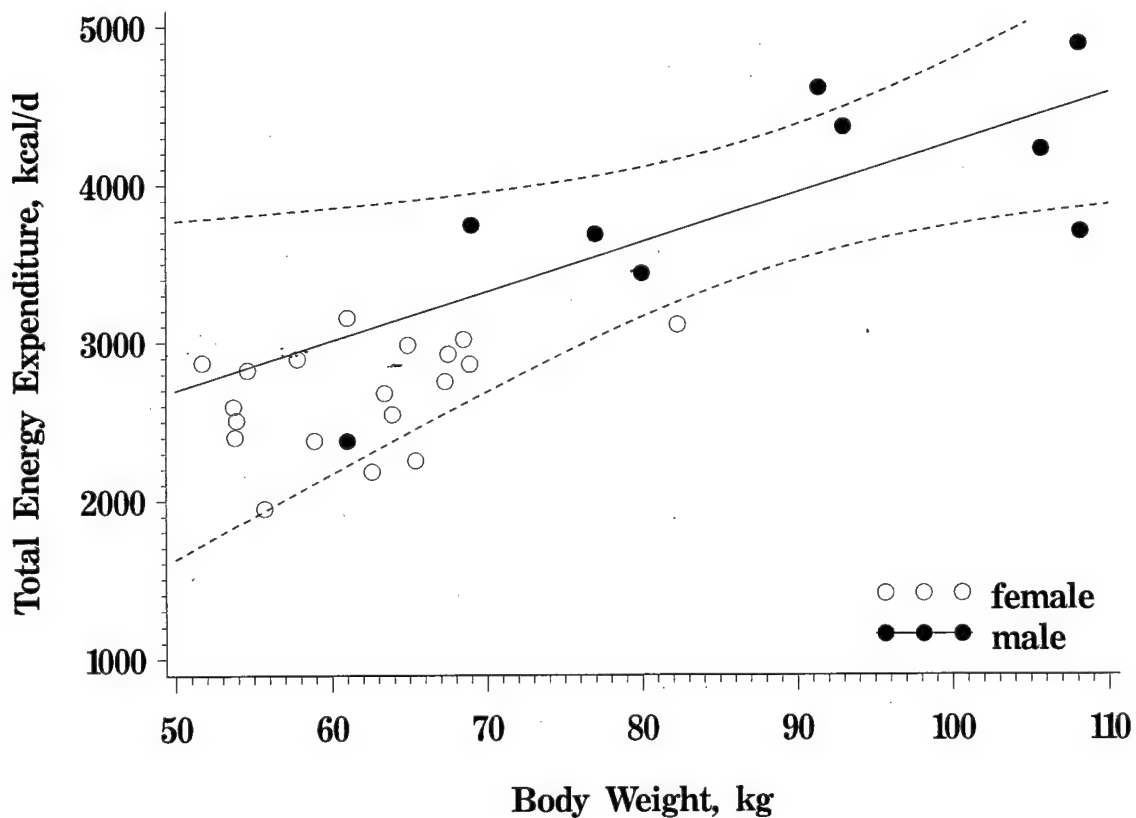
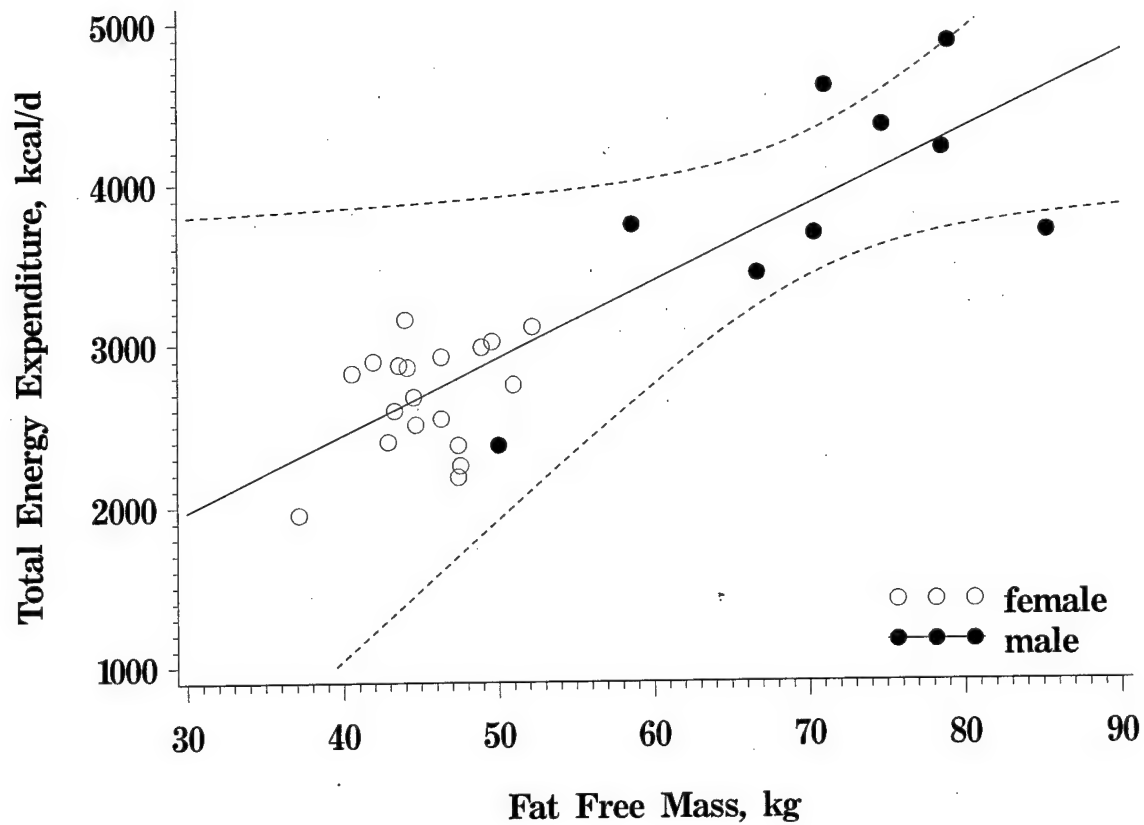
Group	Male		Female	
	N	Mean \pm STD	N	Mean \pm STD
PreFTX				
A	1	3300	5	2220 \pm 448
M	6	3150 \pm 946	7	2372 \pm 426
M1	1	3870	4	2614 \pm 473
S	1	2729	3	1848 \pm 248
FTX				
A	1	3709	4	2332 \pm 373
M	6	3880 \pm 872	6	2872 \pm 229
M1	1	4261	4	2940 \pm 268
S	2	4174 \pm 431	3	2781 \pm 320

A more thorough effort was undertaken to adjust energy expenditures for differences in body weight between the men and women. A more appropriate method than simply dividing energy expenditure by body weight is to use body weight or fat free mass as covariance analysis of variance to adjust for differences in body size. In addition to body weight or fat free mass, we included job classification group, since these were not entirely balanced between the males and females. These adjustments to energy expenditure are given below in Table 3. The adjustments for body weight are somewhat suspect, because most soldiers were in BDUs (Battle Dress Uniform) during the initial weight and we had to adjust the body weights. Therefore, adjustments using FFM (measured from isotope dilution as part of the DLW method) are more likely to be accurate. In addition, although the energy data have been broken down into the short pre-FTX (3 days) and the FTX, the data from the entire period, using linear regression to calculate elimination rates will be the more accurate measure of energy expenditure. Energy expenditure, adjusted for differences in body size and imbalances in MOS group, tended to be higher during the FTX in men compared to women. During the entire period, energy expenditure was significantly higher when adjusting for body weight (which was somewhat suspect) but not when adjusting for fat free mass.

Table 3. Energy expenditures adjusted for differences in body size using covariance analysis or variance.

Adjustments	Female	Male
FTX		
Body Weight	2983 \pm 120	3507 \pm 186
Body Weight + Group	2987 \pm 114	3500 \pm 175*
FFM	3058 \pm 160	3364 \pm 266
FFM + Group	3072 \pm 151	3337 \pm 251
Pre-FTX		
Body Weight	2393 \pm 116	2819 \pm 191
Body Weight + Group	2396 \pm 118	2812 \pm 195
FFM	2531 \pm 140	2526 \pm 254
FFM + Group	2547 \pm 143	2492 \pm 260
Entire period, by linear regression		
Body Weight	2907 \pm 106	3398 \pm 176*
Body Weight + Group	2912 \pm 101	3385 \pm 168*
FFM	3031 \pm 132	3135 \pm 240
FFM + Group	3046 \pm 124	3102 \pm 226

Another way to examine energy expenditure is to plot the individual energy expenditure data points versus fat free mass or body weight. It is clearly shown in the top figure comparing energy expenditure to FFM, that the male and female soldiers fall along the same regression line.



In the bottom graph, comparing energy expenditure to body weight, although the majority of the female data points fall below the regression line, most are within the 95% confidence intervals, indicating that energy expenditure adjusted for body weight was not different between men and women.

2) Activity monitor data has been analyzed.

There were no significant differences in Actigraph activity data between males and females. Time spent awake and during sleep, as well as activity events were nearly identical between men and women. The mean daily counts tended to be slightly higher in women (141 vs 131), while the activity events greater than 4 minutes and mean counts during activity tended to be higher in men (5.4 vs 4.6 and 182 vs 130, respectively).

Table 4. Actigraph activity data.

	Females	Males	p
Mean Counts	141 ± 3.3	131 ± 5.5	0.14
Wake, minutes	854 ± 18	850 ± 30	0.90
Sleep, minutes	445 ± 17	489 ± 28	0.19
Sleep latency	26.3 ± 5.5	34.6 ± 9.4	0.45
Activity events	10.2 ± 0.8	10.2 ± 1.4	0.98
Mean Counts, during activity events	130 ± 21	182 ± 36	0.22
Activity events > 5 minutes	4.6 ± 0.3	5.4 ± 0.5	0.16

Data from the activity monitors was used to develop models to approximate energy expenditure measured by DLW. The first model used calculated RMR (based on FFM, (12) multiplied by waking minutes and the mean activity counts (divided by 100, which approximates a multiple of RMR) plus calculated RMR times sleeping minutes, with the sum divided by 1440 minutes/d. In addition, a further activity factor was added using the activity events multiplied by the mean activity counts during activity events, multiplied by weight, and finally divided by 100. The second model was much simpler, estimating activity by multiplying body weight by activity events and the mean activity counts during activity events, divided by 100, then adding RMR. The model fit (r^2 and p) and energy expenditure for females and males is given below. Although the mean values are very close to the DLW values for energy expenditure, the models explain only 55 and 65 % of the variance. Therefore, further work is needed before Actigraph data can be used to estimate energy utilization.

Model 1

$$\frac{(RMR \times Wake \times mean / 100 + RMR \times Sleep)}{1440} + Activity\ Events \times mean\ during\ activity \times weight / 100$$

Model 2

Activity Events x Mean Activity Counts During activity x weight / 100 + RMR

	r^2	p	Females	males
Model 1	0.55	0.0001	2890 \pm 134	4012 \pm 227
Model 2	0.65	0.0001	2610 \pm 100	3674 \pm 169
DLW			2678 \pm 117	3864 \pm 192

MARINE RECRUIT CRUCIBLE STUDIES

We were very fortunate that the opportunity arose to conduct energy expenditure studies in Marine Recruits undergoing the grueling 54.4 hour Crucible event conducted at Parris Island, South Carolina. This gave us the opportunity to study very high energy expenditures in men and women undergoing the same intense training program. The USARIEM group was asked to conduct cold weather studies in January and February, and I was able to join the team as this project fit perfectly with the aims of this grant. Those individuals who were involved in collecting the data in the field included: James DeLany - PBRC; John Castellani, James Moulton, Kate OBrien, Bill Santee - USARIEM. Since the lead time on the January study was very short, we were not able to use any of the activity monitoring devices. However, we were able to use both the actigraphs, and the new foot contact monitors during the second iteration of the Crucible Studies. Volunteer recruitment was conducted as described under the first field study. The general and detailed study protocols are given below.

A. STUDY DESIGN/CONDUCT

1. Energy expenditure studies in a subset during two Crucible Studies
 - a) 15 men
 - b) 10 women
2. Jan-98 Study
 - a) Doubly labeled water
 - b) Weather data
 - c) Intake measurements
3. Feb-98 Study
 - a) DLW
 - b) Actigraph data
 - c) Foot contact monitor data
 - d) Weather data
 - e) Intake measurements

- Protocol

»Baseline Urine Wednesday afternoon

»DLW dose Wednesday afternoon

»0200 Thursday Urine

»2300 Thursday / 0400 Friday Urine

»2300 Friday Urine

»0800 Saturday Urine

In addition, a considerable amount of weather information was gathered throughout the studies. Dietary intake was estimated by having the participants save all Meals Ready to Eat (MRE) wrappers in plastic bags, as well as writing any other food eaten, such as the fresh fruits and hot wets that were also provided. The empty wrappers and other foods written down were then used to estimate food intake throughout the study. This process was made somewhat easier because the soldiers only received two MREs throughout the study.

B. PROGRESS

Isotope analyses have been completed are calculations completed. The calculations for this study were more completed than those for the first field study because the participants in this study were under fed considerably, and therefore used substantial body stores to make up the caloric deficit. This is important, because in the calculation of energy expenditure from the calculated CO₂ production, one uses a caloric equivalent of CO₂ based on the substrates utilized during the study. Normally, during weight maintenance, that would be equivalent to the dietary intake. However, when substantial body stores are also used for energy, this must be taken into account. The calculations for the food quotient (FQ) used for the DLW calculations are given below. The body weight loss data is given in the Appendix.

Parris Island - FQ Calculations - Men

Assume 300g glycogen, 80% fat

		hours	kcal/d	EE total	Intake	Deficit	Fat	Protein	Carb.	
		54.4	6300	14283	3239	11044	7875	1969	1200	
							875	492	300	
							per g substrate			
		Substrate (g)					Liters	total	CO2	O2
		diet	body	total	CO2	O2	/L CO2	kcal	formed	used
Prot	101	492.2	593	459	573	5.579	2561	0.774	0.966	
CHO	448	300.0	748	620	620	5.047	3130	0.829	0.829	
Fat	123	875.0	998	1424	2015	6.629	9441	1.427	2.019	
				2503	3208			15132		
		RQ		0.780	kcal/L CO2		6.045			

Parris Island - FQ Calculations - Women

Assume 240g glycogen, 80% fat

Assume 20 g glycogen, 100 kcal									
hours		kcal/d	EE total	Intake	Deficit	Fat	Protein	Carbohydrate	
54.4		4770	10814	2580	8234	5819	1455	960	
						647	364	240	
							per g substrate		
Substrate (g)						Liters	total	CO2	O2
	diet	body	total	CO2	O2	/L CO2	kcal	formed	used
Prot	98	363.7	462	357	446	5.579	1994	0.774	0.966
CHO	400	240.0	640	531	531	5.047	2678	0.829	0.829
Fat	116	646.6	763	1088	1540	6.629	7214	1.427	2.019
				1976	2516	11885			
RQ		0.785		kcal/L CO2		6.014			

The energy expenditures for each of the Crucible studies is given in the following table. The detailed data is presented in the Appendix. As in the previous field study presented above, energy expenditure was significantly higher in men than women. However, when simply dividing by body weight energy expenditures were similar, particularly in the second Crucible study. Of interest, and as expected, energy expenditures were much higher in the Crucible studies compared to the combat support hospital study. Energy expenditure in women was nearly 2000 kcal per day higher in this study, and nearly 1000 kcal/d higher than the men in the previous study. Further data analyses need to be performed.

EE, kcal/d		EE, kcal/kg/d	
Mean	SD	Mean	SD

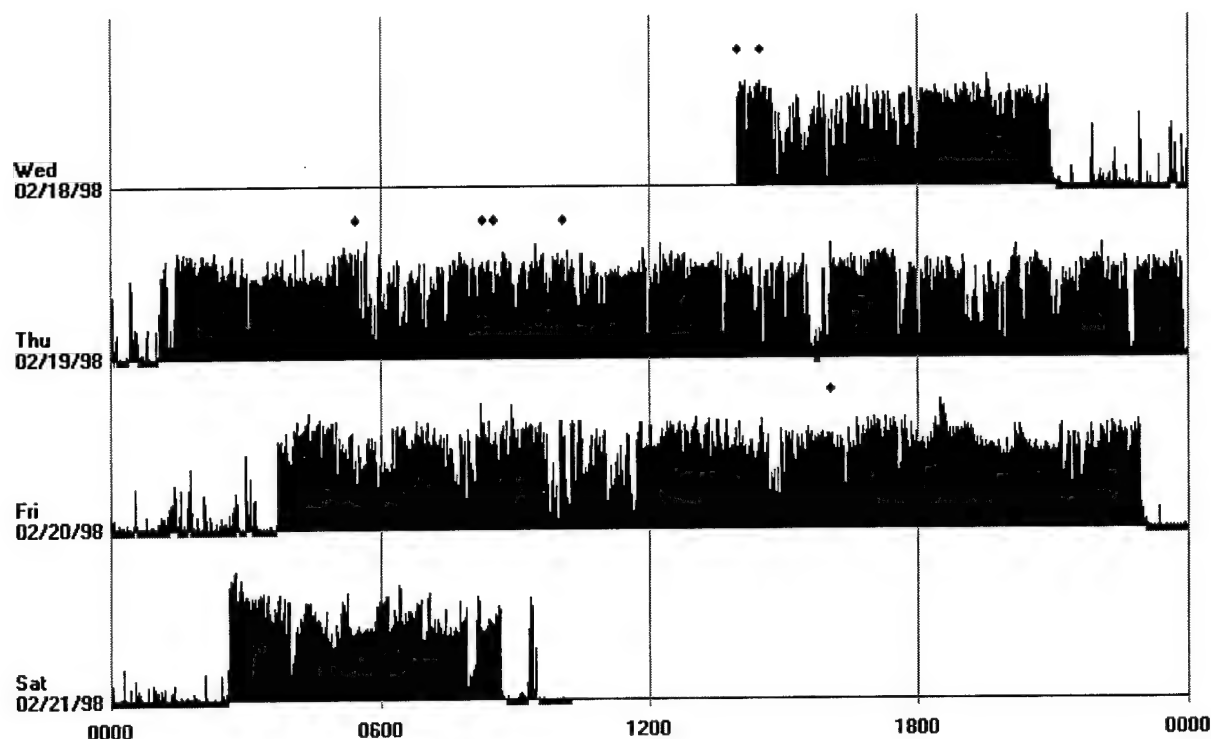
JANUARY CRUCIBLE

Men	6448	868	91.1	15
Women	4800	576	83.5	15

FEBRUARY CRUCIBLE

Men	5787	1085	80.8	18
Women	4653	725	80.8	18

Analysis of the Actigraph data has begun. All of the raw data have been crunched, but no further analyses have been conducted. The figure below depicts a typical output for the Crucible studies, indicating the little time for sleep in these studies.



7. CONCLUSIONS

Overall the field studies have gone very smoothly. We hope that the rest of the studies go as well. When adjusting for differences in body size, the energy expenditure of men and women were similar in the Combat Support Hospital study. Energy expenditures during the short term Crucible studies were very high, possibly some of the highest energy expenditures we will observe. The Crucible studies will provide an excellent paradigm to examine energy expenditures between men and women because all recruits underwent the essentially the same activities and were on the same sleep/wake regimen. When all of the studies are complete, and we can combine all of the data covering a wide range of expenditures, we will be better able to make final conclusions about energy requirements of female military personnel compared to men.

Planning for the shipboard studies is underway, with a meeting scheduled in San Diego next month to work out the protocol and begin the process for human subject review. We have also begun discussions to conduct studies in Marines during basic training, basic training at the Great Lakes Naval Training Center and/or basic training in an Army unit with women.

Reference List

1. DoD. Military Manpower Statistics - June 30, 1993. U.S. Department of Defense. Washington, DC: Washington Headquarters Services, Directorate for Information Operations and Reports AD A273 367. 1993. Washington, DC. 1993.
2. King, N., Arsenault, J. E., Champagne, C., Mutter, S. H., Murphy, C. M., Westphal, K. A., and Askew, E. W. Nutritional Intake of Female Soldiers During the U.S. Army Basic Combat Training. AD A283 601. 1997. Natick, MA, U.S. Army Research Institute of Environmental Medicine. Technical Report T94-17.
3. Departments of the Army, the Navy, and the Air Force, and Headquarters. Nutrition Allowances, Standards, and Education. 1985. Washington, DC. AR 40-25/NAVMEDCOMINST 10110.1/AFR 160-95.
4. Friedl KE, Westphal KA, Marchitelli LJ. Reproductive status and menstrual cyclicity of premenopausal women in Army basic combat training. *FASEB J* 1995;9:292(abst)
5. Schoeller DA, van Santen E, Peterson DW, Dietz W, Jaspan J, Klein PD. Total body water measurement in humans with ^{18}O and ^2H labeled water. *Am.J.Clin.Nutr.* 1980;33:2686-2693.
6. Schoeller DA. Measurement of energy expenditure in free-living humans by using doubly labeled water. [Review]. *J.Nutr.* 1988;118:1278-1289.
7. DeLany JP, Schoeller DA, Hoyt RW, Askew EW, Sharp MA. Field use of D_2^{18}O to measure energy expenditure of soldiers at different energy intakes. *J.Appl.Physiol.* 1989;67:1922-1929.
8. Racette SB, Schoeller DA, Luke AH, Shay K, Hnilicka J, Kushner RF. Relative dilution spaces of ^2H - and ^{18}O -labeled water in humans. *Am.J.Physiol.* 1994;267:E585-90.
9. Hoyt RW, Knapik JJ, Lanza JF, Jones BH, Staab JS. Ambulatory Foot Contact Monitor to Estimate Metabolic Cost of Human Locomotion. *J.Appl.Physiol.* 1994;76:1818-1822.
10. Hoyt RW, Jones TE, Stein TP, et al. Doubly labeled water measurement of human energy expenditure during strenuous exercise. *Journal.of.Applied.Physiology.* 1991;71:16-22.
11. Lieberman HR, Wurtman JJ, Teicher MH. Circadian rhythms of activity in healthy young and elderly humans. [Review] *Neurobiology.of.Aging* 1989;10:259-265.
12. Luke A, Schoeller DA. Basal metabolic rate, fat-free mass, and body cell mass during energy restriction. [Review]. *Metabolism* 1992;41:450-456.

Appendix 1. Body weight changes and dietary intake for January Crucible Study

January 98

Men, g 101 123 448

Subj#	Age	Weight			subj#	Total
		Initial	Final	Loss		
1	19	73.2	68.5	4.7	1	5387
2	19	65.2	63.0	2.2	2	1980
3	19	64.4	62.3	2.1	3	2084
4	19	86.0	84.0	2.0	4	2824
5	18	81.3	77.5	3.8	5	2628
6	22	88.1	84.6	3.5	6	2107
7	19	61.4	57.9	3.5	7	3152
8	19	84.2	79.7	4.5	8	3218
9	25	76.2	74.6	1.6	9	3350
10	18	67.3	64.6	2.7	10	4580
11	26	80.9	76.8	4.1	11	3293
12	19	72.0	68.9	3.1	12	5516
13	18	66.4	63.2	3.2	16	2149
14	19	81.2	76.6	4.6	17	1988
15	19	73.2	70.2	3.0	18	3048
16	19	53.4	51.5	1.9	19	3167
17	18	63.4	62.2	1.2	21	2403
18	18	62.1	60.5	1.6	23	2200
19	20	68.5	67.4	1.1	25	3187
20	19	46.7	45.6	1.1		
21	19	66.1	64.4	1.7		
22	18	72.9	70.8	2.1		
23	20	44.9	43.4	1.5		
24	19	44.7	42.8	1.9		
25	21	58.7	57.1	1.6		

Women, g	92	105	324
	PRO	FAT	CHO
3343	544	1595	3388
	221	840	945
	206	941	1255
	447	964	1585
	361	1093	1196
	398	856	880
	590	1253	1354
	318	1241	1748
	372	1135	1888
	430	1431	2778
2592	363	1019	1946
	504	1367	3811
	336	588	1273
	269	968	791
	303	1244	1577
	312	1040	1832
	440	1040	960
	340	736	1169
	330	979	1809
Men	PRO	FAT	CHO
	396	1145	1898
Women	99	127	474
	333	942	1344
	83	105	336

Appendix 2. Body weight changes and dietary intake for February Crucible Study

February 98

	Age	Weight			subj#	Total		PRO	FAT	CHO
		Initial	Final	Loss						
1	21	72.2	68.0	4.2	1	2995	3135	337	1041	1648
2	19	70.9	67.4	3.5	2	2951		371	1118	1517
3	19	80.0	76.2	3.8	3	3466		425	1122	1929
4	21	80.7	77.5	3.2	4	3919		380	1232	2347
5	20	87.0	84.3	2.7	5	4068		472	1183	2509
6	19	72.7	69.9	2.8	6	3485		602	1333	1603
7	18	67.9	65.6	2.3	7	3443		418	1256	1820
8	21	70.4	67.6	2.8	8	1995		367	619	1008
9	18	85.7	82.6	3.1	9	3195		443	1187	1595
10	19	75.7	73.3	2.4	10	3943		549	1133	2268
11	19	68.2	65.3	2.9	11	1472		131	624	770
12	20	69.1	66.4	2.7	12	2056		436	941	693
13	24	60.8	57.5	3.3	13	3695		459	1238	2007
14	22	65.7	62.9	2.8	14	4716		550	1581	2669
15	22	66.6	63.7	2.9	15	1629		202	535	902
16	19	65.9	64.7	1.2	16	2392	2568	417	700	1282
17	19	65.6	62.6	3.0	17	5227		881	1927	2489
18	18	61.6	60.0	1.6	18	1218		137	483	623
19	23	53.5	51.5	2.0	19	2933		507	1260	1224
20	28	65.5	65.0	0.5	20	2218		441	922	894
21	19	62.2	60.3	1.9	21	1939		249	699	1019
22	23	46.5	44.7	1.8	22	3831		510	1325	2020
23	18	53.6	51.8	1.8	23	2616		441	1049	1175
24	20	59.5	58.5	1.0	24	2022		283	695	1079
25	18	56.9	54.7	2.2	25	1284		160	475	673

	PRO	FAT	CHO
Men	409	1076	1686
	102	120	421
Women	403	954	1248
	101	106	312

Appendix 3. Energy expenditure for Crucible Studies.

Preliminary Energy Expenditure (EE): Parris Island 1/98 Crucible Study

S#	TBW	KO	KD	EE kcal/d	Men		Body wt.	kcal/kg
#1	48.07	0.21465	0.16702	6937	Mean	SD	70.1	99.0
#2	41.35	0.20148	0.15044	6532	6448	868	69.2	94.5
#3	45.54	0.16409	0.12567	5350			78.1	68.5
#4	52.21	0.16848	0.12623	6817			79.1	86.2
#5	55.99	0.26230	0.21650	7407			85.7	86.5
#6	60.98	0.15041	0.11566	6464			71.3	90.7
#7	39.81	0.12498	0.09135	4180			66.8	62.6
#8	54.18	0.16463	0.12208	7154			69.0	103.7
#9	49.38	0.17743	0.13257	6854			84.2	81.5
#10	43.79	0.14605	0.10587	5510			74.5	74.0
#11	55.67	0.14574	0.10458	7199			66.8	107.9
#12	44.37	0.13718	0.09535	5888			67.8	86.9
#13	46.24	0.12346	0.07934	6585			59.2	111.3
#14	53.10	0.16296	0.12094	6923	Women		64.3	107.7
#15	52.12	0.16778	0.12497	6912	Mean	SD	65.2	106.1
#16	30.99	0.15145	0.09686	5467	4800	576	65.3	83.7
#17	33.61	0.19337	0.14931	4507			64.1	70.3
#18	33.93	0.20279	0.15102	5444			60.8	89.5
#19	25.10	0.57418	0.51009	4042			52.5	77.0
#20	28.41	0.15200	0.10736	4006			65.3	61.4
#21	37.17	0.14950	0.10536	5185			61.3	84.7
#22	39.09	0.11135	0.06944	5316			45.6	116.6
							83.5	15

#23	27.26	0.17041	0.11909	4433
#24	28.17	0.15705	0.10773	4423
#25	33.77	0.15591	0.10765	5179

52.7 84.1
59.0 75.0
55.8 92.8

NOTE: The FQ used to convert CO2 production to EE estimated from food intake and body stores used for energy.

Average TBW estimated as 1/2 average body weight loss - body stores used for energy.

Preliminary Energy Expenditure: Parris Island 2/98 Crucible Study,

S#	TBW	KO	KD	EE kcal/d	Body Wt	kcal/kg
#1	44.74	0.19029	0.14003	7001	70.9	98.8
#2	44.07	0.15318	0.10774	6331	64.1	98.8
#3	46.73	0.21904	0.17623	5920	63.4	93.4
#4	48.18	0.15348	0.11519	5695	85.0	67.0
#5	54.75	0.16711	0.12028	8048	79.4	101.4
#6	42.45	0.15360	0.10829	6076	86.4	70.4
#7	44.14	0.24906	0.19983	6446	59.7	108.1
#8	39.09	0.24314	0.21121		82.0	
#9	49.66	0.12981	0.10262	4050	75.4	53.7
#10	47.38	0.22328	0.18594	5058	66.0	76.7
#11	45.59	0.12737	0.09585	4431	78.9	56.2
#12	44.64	0.13038	0.09497	4942	70.5	70.1
#13	40.53	0.09639	0.06055	4709	64.8	72.7

Men	
Mean	SD
5787	1085

80.8	18
------	----

#14	41.36	0.15649	0.11223	5753	Women		78.9	72.9
#15	40.38	0.17042	0.11912	6562	Mean	SD	71.7	91.5
#16	38.64	0.14845	0.10336	5524	4653	725	52.5	105.3
#17	37.71	0.20600	0.15706	5656			62.8	90.1
#18	33.15	0.14191	0.09969	4426			61.3	72.2
#19	19.51	0.65661	0.58424	3530			68.0	51.9
#20	35.61	0.20896	0.16092	5214			46.2	113.0
#21	32.71	0.17785	0.13030	4850			65.3	74.3
#22	27.16	0.21229	0.15222	5127			71.9	71.4
#23	30.05	0.12875	0.08804	3897			44.2	88.3
#24	34.08	0.17554	0.13562	4139			43.8	94.6
#25	31.72	0.13451	0.09317	4164			57.9	71.9

NOTE: The FQ used to convert CO2 production to EE estimated from food intake and body stores used for energy.

Average TBW estimated as ½ average body weight loss - body stores used for energy.

83.3	18
------	----